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Evaluation of the Technicon Bound Uricase Method for the Determination of Uric Acid in Urine

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Summary: We have evaluated the Technicon bound uricase method for the determination of uric acid in urine with the AutoAnalyzer II. The general analytical characteristics of the method, and the effect of urine on the immobilized uricase nylon tube reactor were investigated.

The method was found to be linear up to 7.0 mmol/l with aqueous standards and up to 5.0 mmol/l with urine samples.

Between-days imprecision had a coefficient of variation (CV) of 1.6% for values of about 1.2 mmol/l, and 0.8% for values above 3.5 mmol/l. Within-run imprecision gave a CV of 0.7% for values of 1.07 and 2.95 mmol/l and a CV of 0.4% for a value of 4.77 mmol/l. The mean analytic recovery was 99.1% (range 93.8–103.0%). The sample interaction was 0.9%.

The correlation with the phosphotungstate method and the manual Dutch standard method was good, but the enzymatic values were 20% lower than the phosphotungstate values. Storing the immobilized uricase nylon tube reactor at 4 °C, when not in use, prolonged the lifetime by nearly 50%. Urine samples were not different from aqueous uric acid standards in their effect on the stability of the uricase nylon tube reactor.

Evaluation des Technicon-Verfahrens mit immobilisierter Uricase zur Harnsäurebestimmung im Harn

Zusammenfassung: Das Technicon-Verfahren mit immobilisierter Uricase zur Harnsäurebestimmung im Harn mit dem AutoAnalyzer II wurde geprüft. Die allgemeinen analytischen Charakteristika der Methode und die Wirkung von Harn auf die an Nylon-Schlauch immobilisierte Uricase wurden untersucht.

Die Methode zeigte mit wäßrigen Standards bis zu 7 mmol/l, mit Harnproben bis zu 5 mmol/l Linearität.

Die Prüfung der Präzision von Tag zu Tag ergab Variationskoeffizienten von 1,6% für Werte von etwa 1,2 mmol/l und 0,8% für Werte über 3,5 mmol/l; innerhalb der Serie ergaben sich Variationskoeffizienten von 0,7% für Werte von 1,07 und 2,95 mmol/l und von 0,4% für einen Wert von 4,77 mmol/l. Die Wiederfindung betrug im Mittel 99,1% (Bereich 93,8–103%). Die Verschleppung betrug 0,9%. Die Korrelation der Ergebnisse der Phosphorwolframsäuremethode mit denen der manuellen Niederländischen Standardmethode war gut, jedoch ergab die enzymatische Methode 20% niedrigere Werte. Die Aufbewahrung des Nylon-Schlauches mit der immobilisierten Uricase bei 4 °C verlängerte die Lebenszeit um etwa die Hälfte. Die Wirkungen von wäßrigen Harnsäurestandards und Harnproben auf das an Nylon-Schlauch immobilisierte Enzym unterschieden sich nicht.

Introduction

To determine uric acid in serum and urine two methods are used: the determination based on the reducing action of uric acid and the enzymatic determination using uricase (urate oxidase EC 1.7.3.3). In many clinical chemical laboratories (44.8% of the 1221 laboratories participating in the Wellcome quality control program and 36.1% of the 169 laboratories in the Netherlands participating in our National quality control program, according to the last published data) the first method is used, with alkaline phosphotungstate as oxidizing agent, first described in 1912 by *Folin & Denis* (1). The method is subject to interference from other reducing compounds, such as drugs and their metabolites (2). The enzymatic method, performed by *Praetorius & Poulsen* (3) as an ultraviolet test with direct photometry of the decrease of uric acid at 293 nm, is very specific. However it is not easy to auto-

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mate determinations at this wavelength. Therefore most automated enzymatic uric acid procedures incorporate the determination of the liberated hydrogen peroxide with the aid of a chromophore. The selection of a proper chromophore is essential for the ultimate specificity and simplicity of the method (4, 5). Using a continuous flow system, the enzymatic uric acid determination is very expensive, compared to the phosphotungstate method. This disadvantage is solved by introducing the immobilized enzyme nylon tube reactor for the routine determination of uric acid in serum with uricase as the immobilized enzyme. The results with home made nylon tube reactors (6, 7) and with commercially available nylon tube reactors (8, 9) are said to be reliable.

We were interested in studying this new methodology in more detail, especially with respect to the determination of uric acid in urine. Little information was available, so we had to look at the general analytical characteristics of the method as well as the effect of urine on the immobilized uricase nylon tube reactor. Furthermore we compared this method with two others, i.e. the Technicon phosphotungstate method, which is in routine use in our laboratory, and the manual Dutch standard method.

Materials and Methods

Equipment

A Beckman DU-2 spectrophotometer was used for all absorbance measurements performed at 410 nm and 570 nm. This instrument was checked regularly according to *Rand* (10) with respect to wavelength setting (holmium) and absorbance measurement (cobalt sulphate).

Chemicals

Lithium carbonate was purchased from Brocacef, Maarsen, The Netherlands (cat. nr. LI 0354); uric acid was from Merck, Darmstadt, FRG (cat. nr. 817).

Standards

Aqueous uric acid standards, ranging from 1 to 8 mmol/l were prepared according to *Fossati* et al. (5). The standards, when frozen in 25 ml portions at −20 °C, are stable for at least six weeks.

Control urines

Between-days imprecision

The lyophilized control urine I (lot. nr. 1S 111N, Ortho Diagnostics Inc. Rariton, New Jersey 08869) was reconstituted with distilled water and with the 5 mmol/l uric acid standard solution to obtain a low and a high control level. A 1 + 1 mixture of these, provided us with an intermediate concentration.

Within-run imprecision

The lyophilized control urine II (lot. nr. 1S 209A, Ortho) was reconstituted with distilled water and with the 2 mmol/l and 4 mmol/l uric acid standard solutions.

Immobilized uricase nylon tube reactors

We used 3 immobilized uricase nylon tube reactors (= uricase coils[1]):

Uricase coil A was used for the determination of uric acid in aqueous uric acid standards and was kept, filled with recipient diluent, at 4 °C when not in use.

Uricase coil B was used for the determination of uric acid in urine and was stored in the same way as uricase coil A.

Uricase coil C was used for the determination of uric acid in urine and was always kept at room temperature.

The stability of the uricase coils was studied by measuring the absorbance found at 570 nm with the uric acid standard of 5 mmol/l.

The results from urine specimens given in this article were obtained with uricase coil B, unless otherwise stated.

The Technicon bound uricase method

The Technicon bound uricase method for uric acid in serum was used for the determination of uric acid in urine according to the manufacturer's instruction (11). Because of the higher uric acid concentration in urine, we modified the AutoAnalyzer II module by changing the 24"-dialyzer for a 3"-dialyzer and by reducing the sample tube (0.10 ml/mm instead of 0.23 ml/mm).

The Technicon phosphotungstate method

The Technicon phosphotungstate method for uric acid in serum was used for the determination of uric acid in urine according to the manufacturer's instruction (12).

The AutoAnalyzer II module was used with a 21 fold predilution of the urine samples (sample tube 0.10 ml/min and water prediluent tube 2.00 ml/min).

The manual Dutch standard method

For the manual uric acid determination we used the method recommended by the Dutch Standardization Committee on Clinical Chemistry. This procedure is highly comparable to the method of *Kageyama* (13).

Before analysing, the urine samples were diluted eleven times with distilled water.

Samples

All urine samples were analysed in duplicate and generally with all methods on the same day. Otherwise the samples were frozen (−20 °C). After thawing the samples, they were kept in a 50 °C water bath for about 10 minutes before they were analysed.

Between-days imprecision

The between-days imprecision was estimated according to the NCCLS procedure, described in "The protocol for establishing performance claims for clinical chemical methods" (14).

Results

Imprecision study

The results of the between-days imprecision and the within-run imprecision of the bound uricase method are given in table 1.

[1]) Non standard abbreviation used: Immobilized-enzyme nylon tube reactor is a generic name given to nylon-tube-supported enzymes; they are manufactured for continuous flow analysis as a coil. For brevity in this paper we use the name uricase coil.

Tab. 1. Results of imprecision studies with the bound uricase method.

Between-days imprecision

Concentration level	Average (mmol/l)	S.D. (mmol/l)	CV (%)	n
Low	1.22	0.02	1.6	20
Intermediate	3.53	0.03	0.8	17
High	5.70	0.05	0.8	17

Within-run imprecision

Concentration level	Average (mmol/l)	S.D. (mmol/l)	CV (%)	n
Low	1.07	0.01	0.7	20
Intermediate	2.95	0.02	0.7	20
High	4.77	0.02	0.4	20

Tab. 2. Results of recovery studies with the bound uricase method.

Sample no.	Initial value (mmol/l)	Added uric acid (mmol/l)	Final value (mmol/l)	Recovery (%)
01	0.45	3.29	3.72	99.5
02	0.61	3.21	3.77	98.7
03	1.10	2.97	3.93	95.3
04	1.59	2.73	4.15	93.8
05	1.67	2.69	4.22	96.8
06	1.86	2.59	4.34	97.5
07	3.05	1.99	5.10	103.0
08	4.65	1.19	5.90	101.0
09	4.89	1.08	5.86	98.2
10	5.48	0.78	6.38	101.9
11	1.02	3.01	3.97	98.5
12	1.02	2.01	3.04	100.3
13	1.02	1.20	2.25	101.4
14	1.02	0.56	1.60	101.3

Linearity

Linearity was checked with an aqueous uric acid standard of 8 mmol/l. As is shown in figure 1 a deviation of linearity is found at concentrations above 7 mmol/l. We checked this upper limit of linearity with urine samples, by diluting 77 urines two times with distilled water, to see if the same amount of uric acid could be found. As can be seen in figure 2 this is not the case. We found in urine a deviation of the linearity of about 4% at the level of 5 mmol/l and of about 6% at the level of 7 mmol/l.

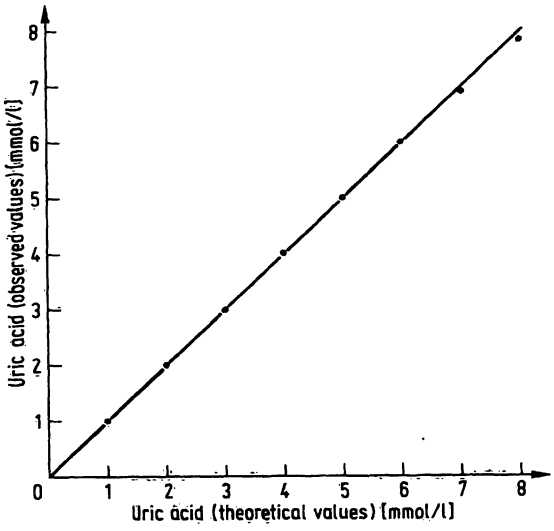

Fig. 1. Linearity of the bound uricase method. Curve constructed with aqueous standards.

Recovery

Different volumes of a uric acid standard prepared in urine were added to eleven different urines with uric acid concentrations ranging from 0.45–5.48 mmol/l.

Table 2 shows the results of these recovery experiments. The mean analytic recovery was found to be 99.1%.

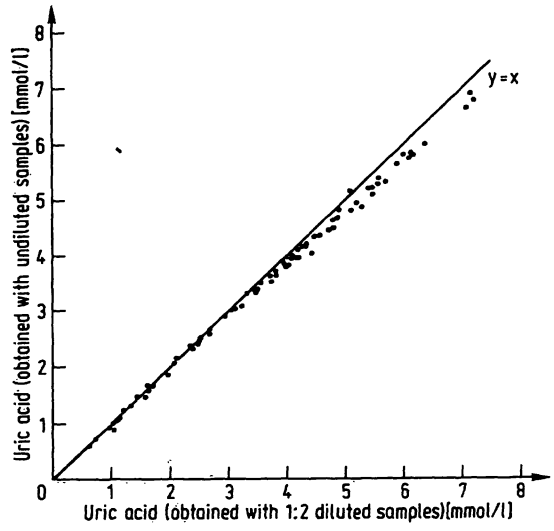

Fig. 2. Linearity check by diluting the urine samples twice with distilled water.

Split-sample comparison

Figure 3 gives the results of the comparison of the uric acid concentrations found in various urine samples with the bound uricase method and the phosphotungstate method.

Figure 4 shows the results if the comparison is made with the Dutch standard method.

Sample interaction

The sample interaction (carry over) was determined according to *Broughton* et al. (15). Using their formula of

$$\frac{b_1 - b_3}{a_3 - b_3} \times 100\%$$

we found a carry over of 0.9% (mean of three determinations: 0.8%, 0.8% and 1.0%).

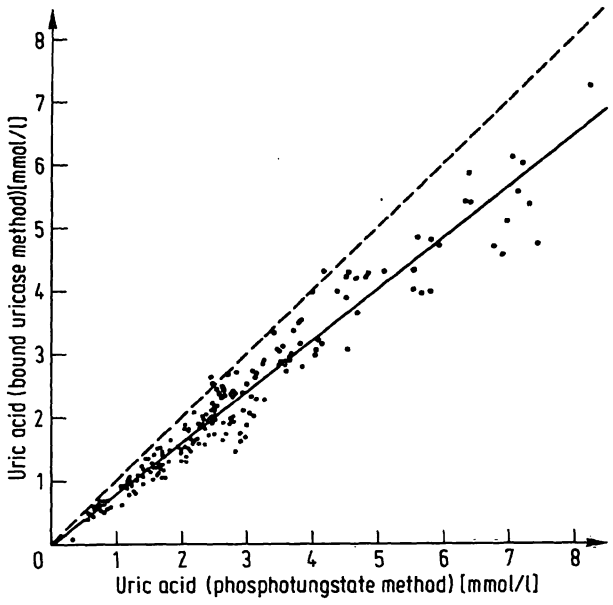

Fig. 3. Split sample comparison between the bound uricase method (y-axis) and the phosphotungstate method (x-axis).
Dashed line represents the line y = x.
Solid line represents the linear regression line:
y (uricase coil) = – 0.01 + 0.81 x (phosphotungstate)
r = 0.973 n = 201

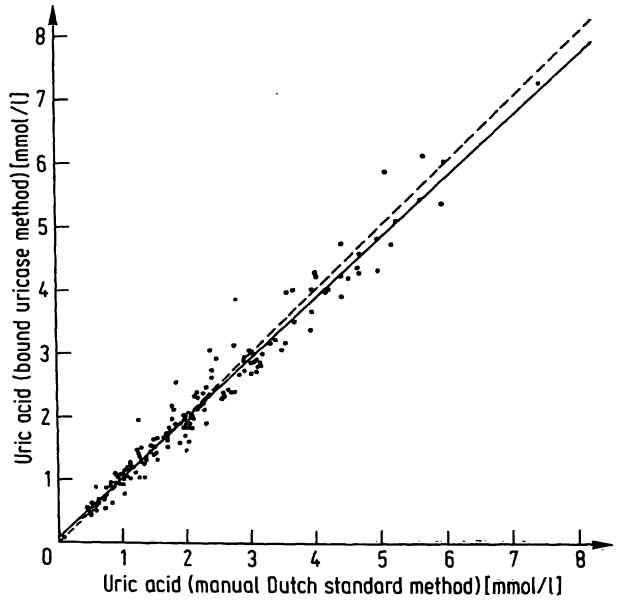

Fig. 4. Split sample comparison between the bound uricase method (y-axis) and the manual Dutch standard method (x-axis).
Dashed line represents the line y = x.
Solid line represents the linear regression line:
y (uricase coil) = 0.08 + 0.95 x (manual method)
r = 0.984 n = 155

The stability of the uricase coil

The course of the stability of the uricase coils A, B and C during the evaluation time of eleven weeks is depicted in figure 5.

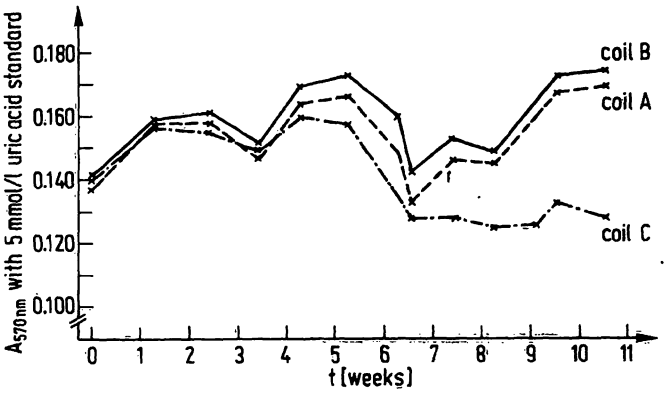

Fig. 5. Stability of the uricase coils A, B, and C. The absorbance of the calibrating material is plotted on the y-axis during an eleven weeks evaluation period.

Discussion

The analytical performance of the determination of uric acid with the Technicon bound uricase method was excellent with regard to the between-days and the within-run imprecision as can be seen in table 1.

The method was found to be linear up to 7 mmol/l uric acid, when tested with aqueous standards (fig. 1).

However, figure 2 shows that it is necessary to dilute those urine samples with an uric acid content of more than 5 mmol/l. We cannot give an explanation for this phenomenon. It is not a serious disadvantage of the method, because the uric acid concentration exceeds 5 mmol/l in only a few urines (3% of a total of about 500 urines tested in this evaluation).

The mean analytic recovery of 99.1% (tab. 2) was satisfactory, although the range (93.8–103.0%) is somewhat broad. The split sample comparisons showed that the results of this continuous flow enzymatic uric acid determination are in agreement with those of the discrete enzymatic Dutch standard method (fig. 4).

The correlation with the phosphotungstate method (fig. 3) was also good, but the enzymatic values were consistently lower (about 20%), which corresponds with the findings of *Henry* et al. (16) and *Gochman & Schmitz* (17). So these results indicate again the well known lack of analytical specificity of the phosphotungstate method for the determination of uric acid.

Monitoring the absorbance at 570 nm of the calibrating material, as a measure for the enzyme stability, we see in figure 5 that the day-to-day variation of the absorbance is considerable. The reason for this variation is not understood, but it was also seen in the evaluation of the Technicon co-immobilized hexokinase/glucose-6-phosphate dehydrogenase method for glucose (18). Both immobilized enzyme methods use instable chemicals, which could be responsible for this variation, but it could also be a phenomenon typical of immobilized enzymes.

Despite this day-to-day variation of the absorbance one can say that the uricase coil, like the co-immobilized enzyme coil from Technicon, shows a relatively constant enzyme activity during the evaluation period. *Werner* et al. (6) and *Sundaram* et al. (7), however, found with their home made nylon tube reactors a decay in enzyme activity once it was installed and used on the analytical system.

The stability of the uricase coils used is guaranteed by the manufacturer for one month under normal operating conditions, storing the coil at room temperature. Figure 5 shows that for intermittent use we found a clear loss of activity after about 7 weeks, which coincided with a loss of linearity with urines and aqueous standards (uricase coil C). By storing the uricase coil at 4 °C when not in use, the stability could be prolonged to eleven weeks (uricase coils A and B). But after 10 weeks there was a loss in linearity with the control urines, especially at the high control level. However we did not find a loss in linearity with aqueous standards. This means that the influence of different storage conditions on the lifetime of the uricase coil is not so great as figure 5 presumes. It also shows that it is better to control the stability of the uricase coil with control urines, than with uric acid standards. Nevertheless, on the basis of our results the lifetime of the uricase coil can be prolonged by nearly 50% by storing the uricase coil at 4 °C when not in use.

With both uricase coils A and B we performed about 1600 tests. As can be seen in figure 5 the stabilities of the two uricase coils do not differ significantly. This indicates that urine samples are not different from aqueous uric acid standards in their effect on the stability of the uricase coil.

The price per test with the immobilized uricase coil depends on the number of tests performed with this method. Running the maximal number of tests (about 6000) the bound uricase method is about 3.8 times cheaper than the uric acid determination using soluble uricase. It is then even 1.3 times cheaper than the phosphotungstate method. In our evaluation, performing 1600 tests, the bound uricase method was 1.6 times cheaper than the soluble uricase method.

Finally, we can conclude that the Technicon bound uricase method, developed for the determination of uric acid in serum, is (with a slight change of the manifold to allow for the higher uric acid concentration in urine) a convenient and specific method for the determination of uric acid in urine.

Acknowledgement

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